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Proteomic diversity of high-density lipoprotein explains its association with clinical outcome in patients with heart failure

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Aims

Previously, low high-density lipoprotein (HDL) cholesterol was found to be one of the strongest predictors of mortality and/or heart failure (HF) hospitalisation in patients with HF. We therefore performed in-depth investigation of the multifunctional HDL proteome to reveal underlying pathophysiological mechanisms explaining the association between HDL and clinical outcome.

Methods and results

We selected a cohort of 90 HF patients with 1:1 cardiovascular death/survivor ratio from BIostat-CHF. A novel optimised protocol for selective enrichment of lipoproteins was used to prepare plasma. Enriched lipoprotein content of samples was analysed using high resolution nanoscale liquid chromatography-mass spectrometry-based proteomics, utilising a label free approach. Within the HDL proteome, 49 proteins significantly differed between deaths and survivors. An optimised model of 12 proteins predicted death with 76% accuracy (Nagelkerke $R^2=0.37$, $P<0.001$). The strongest contributors to this model were filamin-A (related to crosslinking of actin filaments) [odds ratio (OR) 0.31, 95% confidence interval (CI) 0.15–0.61, $P=0.001$] and pulmonary surfactant-associated protein B (related to alveolar capillary membrane function) (OR 2.50, 95% CI 1.57–3.98, $P<0.001$). The model predicted mortality

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with an area under the curve of 0.82 (95% CI 0.77–0.87, $P < 0.001$). Internal cross validation resulted in $73.3 \pm 7.2\%$ accuracy.

Conclusion

This study shows marked differences in composition of the HDL proteome between HF survivors and deaths. The strongest differences were seen in proteins reflecting crosslinking of actin filaments and alveolar capillary membrane function, posing potential pathophysiological mechanisms underlying the association between HDL and clinical outcome in HF.

Keywords

Heart failure • High-density lipoprotein • Proteome

Introduction

Several studies showed that lower high-density lipoprotein (HDL) cholesterol was associated with a higher incidence of heart failure (HF),¹ worsening of HF,² and mortality and HF hospitalisation in patients with established HF.^{2–5} We recently studied predictors of clinical outcome in two large European cohorts of patients with HF [A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF)]. Low HDL cholesterol was found to be amongst the strongest independent predictors of death and/or HF hospitalisation.⁶ The exact pathophysiological mechanisms underlying the association between HDL and HF are however unknown.

Technological developments and the use of mass spectrometry have strongly increased our understanding of the proteomic diversity of HDL. Besides the well-known function in reverse cholesterol transport, HDL carries many other constituents. Previous studies have identified over 90 proteins that are consistently co-expressed in variable amounts attached to HDL particles, consisting of apolipoproteins, lipid transfer proteins, enzymes, acute-phase response proteins, complement components, haemostasis proteins and several others. The functions of HDL are therefore multifold: it possesses anti-inflammatory, antioxidant, anti-infective, antithrombotic, and atheroprotective capacities and exerts effects on endothelial function.⁷

We hypothesised that further in-depth investigation of the multifunctional HDL proteome could reveal possible underlying pathophysiological mechanisms of HDL in HF that can help us understand its clinical value in HF. Therefore, this study aims to investigate differences in HDL proteome composition in HF that relate to a worse prognosis in HF.

Materials and methods

Patient population

This study was conducted with patients selected from the BIOSTAT-CHF cohort, which has been described in detail before.⁸ In brief, BIOSTAT-CHF was an investigator-driven multicentre clinical study consisting of 2516 patients, which aimed to identify patients with a poor outcome despite currently recommended treatment using a systems biology approach that incorporates demographics, gender, biomarkers, genetics and proteomics. Patients were included after presentation with either new onset or worsening

HF, which was confirmed by left ventricular ejection fraction $\leq 40\%$ and/or brain natriuretic peptide (BNP) > 400 pg/mL or N-terminal pro BNP (NT-proBNP) > 2000 pg/mL. All patients recruited in BIOSTAT-CHF gave written informed consent to participate in the study. BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki, national ethics and legal requirements, as well as relevant EU legislation. The study was approved by national and local ethics committees.

For this study, 90 patients from BIOSTAT-CHF were selected for a discovery cohort and matched with a 1:1 death/survivor ratio of cardiovascular cause within a follow-up period of 12 months. Patients were furthermore matched for important prognostic criteria. The matching criteria and randomisation procedure are shown in the supplementary material online, *Appendix S1*.

Sample preparation

The detailed sample preparation protocol has been included in the supplementary material online, *Appendix S2*. Lipoproteins were isolated using calcium silicate matrix (commercial name Lipid Removal Agent, Sigma-Aldrich, St. Louis, MO, USA), to separate them from other abundant proteins in plasma which will impair proteomic analysis. Prior to trypsin digestion, disulphide bonds were reduced using tris (2-carboxyethyl) phosphine (TCEP, Sigma-Aldrich), cysteines alkylated by iodoacetamide (IAA, Sigma-Aldrich), following denaturation using ammonium deoxycholate (ADC, 0.5%, deoxycholic acid treated with neat ammonium hydroxide, Sigma-Aldrich), thus allowing trypsin maximum access to the cleavage sites within the protein and preventing renaturation. Trypsin (Sigma-Aldrich) was added and the samples were incubated at 37°C for 16 h. Trypsin digestion was stopped by lowering the pH of the sample with a volume of fully concentrated formic acid (FA, final concentration 1%, Sigma-Aldrich). Salts and other undesired impurities were eliminated by solid phase extraction on EMPORE C18 discs. A semi-pooled sample was created from 10 random samples of the discovery cohort to serve as a quality control (QC) sample. The QC samples were treated equal to the other samples.

Samples were reconstituted in purified water containing FA (0.1%) and spiked with an internal standard, MassPREP™ yeast alcohol dehydrogenase (ADH, Waters Corporation, Manchester, UK), with a known amount of 50 fmol injected for each run; 1 μL of sample was injected. All samples were analysed in triplicate. A QC was run in triplicate after each batch of 10 samples with an injection volume of 1 μL for each single run. In between each patient or QC, washings were performed with three different cleaning mixtures that contained isopropyl alcohol, methanol, and FA, respectively.

Table 1 Baseline characteristics of the BIOSTAT-CHF discovery cohort

Characteristics	Survivors (n = 45)	Deaths (n = 45)	P-value
Age, years	70.0 ± 9.3	69.9 ± 8.8	0.963
Male sex, n (%)	39 (87)	39 (87)	1.000
BMI, kg/m ²	28.2 ± 4.8	27.5 ± 6.2	0.524
NYHA class III/IV, n (%)	39 (87)	35 (78)	0.524
LVEF, %	28.0 ± 5.7	26.3 ± 7.2	0.221
Systolic blood pressure, mmHg	124.5 ± 23.6	118.5 ± 21.3	0.210
Diastolic blood pressure, mmHg	73.8 ± 13.7	72.3 ± 11.3	0.576
Heart rate, b.p.m.	76 ± 20	79 ± 15	0.440
BNP, pg/mL	409.3 (162.0–573.2)	488.4 (230.5–766.5)	0.255
eGFR, mL/min/1.73 m ²	56.3 ± 17.2	53.2 ± 21.1	0.455
Serum creatinine, µmol/L	114.0 (98.0–133.9)	124.0 (99.7–163.5)	0.296
Total cholesterol, mmol/L ^a	3.7 (3.3–4.5)	3.2 (2.8–4.2)	0.070
HDL cholesterol, mmol/L ^b	1.1 (1.0–1.3)	0.8 (0.7–1.1)	0.004
LDL cholesterol, mmol/L ^c	2.2 (1.7–3.2)	1.8 (1.5–2.8)	0.092
Diabetes, n (%)	20 (44)	18 (40)	0.670
Hypertension, n (%)	34 (76)	27 (60)	0.114
Reason for visit, n (%)			1.000
New onset of HF	3 (7)	3 (7)	
Worsening HF	42 (93)	42 (93)	

BMI, body mass index; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, heart failure; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

^aData only available for 54 patients (28 survivors; 26 deaths).

^bData only available for 49 patients (26 survivors; 23 deaths).

^cData only available for 48 patients (25 survivors; 23 deaths).

Nanoscale liquid chromatography-coupled mass spectrometry

Each sample was analysed on a Waters NanoAcquity system (Waters Corporation, Milford, MA, USA). The liquid chromatography system was coupled to a Waters Synapt G2S HDMS (Waters Corporation, Milford, MA, USA). Data were acquired using MassLynx 4.1. Details on the settings and methodology that were implemented are described in the supplementary material online, *Appendix S3*.

Data analysis

Raw data were interrogated by Progenesis QI software (Nonlinear Dynamics, Gateshead, UK) for data analysis. A description of the used search criteria is included in the supplementary material online, *Appendix S3*.

Progenesis QI executed label-free quantification of the identified proteins using the Hi-N3 method as first described by Silva *et al.*⁹ Since the samples were internally spiked, this allowed for both Hi-N relative quantification and Hi-N absolute quantification. Analyses were focused on Hi-N relative quantification, with the Hi-N absolute quantification method serving as a verification back-up if needed.

Statistical analysis

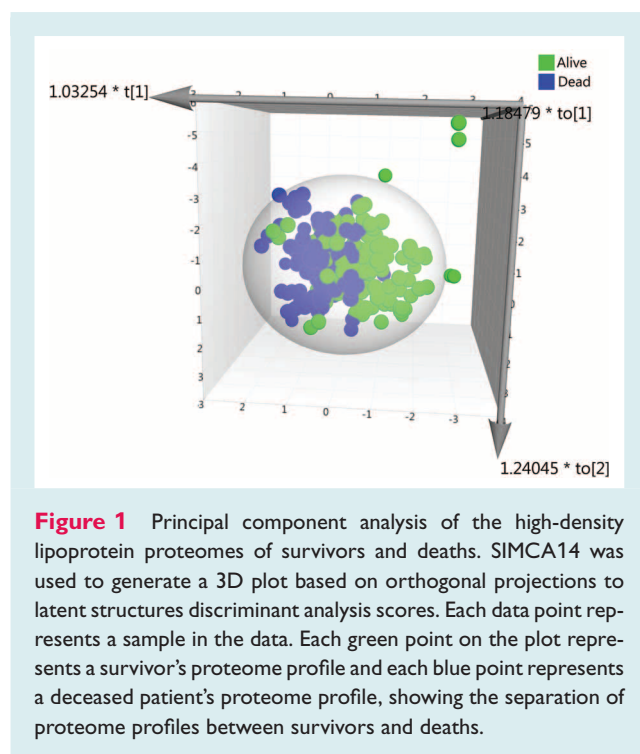
Descriptive statistics were used to examine the relationship of the two groups with clinical variables. Prior to analyses, the distribution of all variables was checked. Data are presented as mean ± standard deviation when normally distributed, as median (interquartile range) for

skewed variables and as frequencies (percentage) for categorical variables. A *P*-value of <0.05 was considered statistically significant. Continuous normally distributed variables were tested with the Student independent *t*-test or analysis of variance (ANOVA), whereas skewed variables were tested using the Kruskal–Wallis *H* test. Categorical variables were tested with Chi-Squared tests. To adjust for multiple testing, a false discovery rate of 1% was implemented for biomarker selection. Different search techniques [logistic regression and orthogonal projections to latent structures-discriminant analysis (OPLS-DA)] were used to establish an optimised model among significant proteins for death prediction. Logistic regression analysis of this optimised model was adjusted for age, sex, trigger event, trigger reason, left ventricular ejection fraction, estimated glomerular filtration rate, and cardiovascular death risk score, which are explained in further detail in *Appendix S1*. Statistical tests were performed with IBM SPSS Statistics version 23. SIMCA version 14 (MSK Umetrics, Sweden) was used to perform principal component analysis and construct the S-plot. Internal cross validation was performed using RapidMiner software version 7.4 for candidate biomarkers. Gene Ontology analysis of proteins was executed using the Protein ANalysis THrough Evolutionary Relationships (PANTHER) Classification System version 11.1.¹⁰

Results

Patient characteristics

Patient characteristics of the two groups are displayed in *Table 1*. Mean age was 70.0 ± 9.3 years in the patients who survived and 69.9 ± 8.8 years in the patients who died. In both groups, most patients were men (87%), most patients had a New



York Heart Association classification of III/IV (87% and 78% for survivors and deaths, respectively), and HDL cholesterol levels were higher in the survivors group [1.1 (1.0–1.3) mmol/L, as opposed to 0.8 (0.7–1.1) mmol/L in deaths, $P = 0.004$]. All other patient characteristics were not significantly different between the two groups. Mean study participation time was 787.0 ± 137.6 days for survivors and 151.7 ± 98.7 days for deaths.

Principal component analysis

The separation of the HDL proteomes of survivors and deaths is indicated in Figure 1. Each point on the plot represents a patient's proteome profile (either deceased or survivor) based on

the patient sample analysis in triplicate. The principal component analysis is also displayed in 2D in the supplementary material online, Figure S1.

HDL proteome biomarkers selection

A total of 647 proteins with quantification were identified from both groups and compared. Out of all reported HDL proteins, as documented by the HDL Proteome Watch (initiated by the Davidson Lab, Cincinnati, OH; database version 14/08/2015), abundances of 49 HDL proteins were significantly different between deaths and survivors (supplementary material online, Table S1). Gene Ontology analysis of these proteins revealed involvement in multiple biological processes, such as cellular processes (e.g. cell communication), biological regulation (e.g. homeostatic process), metabolic processes (e.g. lipid metabolism, protein metabolic process), and response to stimulus (e.g. immune response, response to stress), as shown in the supplementary material online, Figure S2.

An optimised model featuring 12 selected proteins was established, using different search techniques, to predict death. These 12 proteins included coagulation factor X (F10), epoxide hydrolase (EPHX1), filamin-A (FLNA), macrophage-stimulating protein (also called hepatocyte growth factor-like protein), pulmonary surfactant-associated protein B (SFTPB), and serum paraoxonase/arylesterase 1 (PON1) based on multiple regression analysis, and alpha-2-HS-glycoprotein (also called fetuin-A), apolipoprotein A-I (APOA1), apolipoprotein A-II (APOA2), apolipoprotein C-III (APOC3), beta-2-microglobulin, and kallistatin (also called Serpin A4) based on OPLS-DA using a variable importance in projection (VIP) value >1 as cut-off value (data not shown). An overview with details on the proteins is provided in Table 2.

Multiple logistic regression analysis of this set of proteins resulted in an ability to predict death with 76% accuracy (Nagelkerke $R^2 = 0.365$, P -value for the model <0.001). Table 3 shows the contribution of each protein to the prediction model, after correcting for the matching criteria. The proteins that significantly contributed to the prediction of death were SFTPB [odds ratio (OR)

Table 2 Proteins associated with clinical outcome in heart failure as selected by multiple approaches

Protein	Function	Higher or lower in deaths	Fold change	P-value
Alpha-2-HS-glycoprotein	Negative acute phase reactant	Lower	1.18	0.014
Apolipoprotein A-I	Anti-atherogenic, antioxidant, anti-inflammatory	Lower	1.16	<0.001
Apolipoprotein A-II	Anti-atherogenic, antioxidant	Lower	1.28	0.022
Apolipoprotein C-III	Pro-atherogenic	Lower	1.58	0.008
Beta-2-microglobulin	Indicator of immune activation	Higher	1.17	0.045
Coagulation factor X	Coagulation	Higher	1.22	0.021
Epoxide hydrolase 1	Detoxification	Higher	1.17	0.008
Filamin-A	Crosslinks actin filaments	Lower	1.19	0.011
Kallistatin	Antioxidant, anti-inflammatory, anti-fibrotic	Lower	1.27	<0.001
Macrophage-stimulating protein	Stress response, pro-apoptotic	Lower	1.26	0.034
Pulmonary surfactant-associated protein B	Assembly of pulmonary surfactant	Higher	1.39	0.006
Serum paraoxonase/arylesterase 1	Anti-atherogenic, antioxidant, anti-inflammatory	Lower	1.20	0.006

Table 3 Multiple logistic regression model for prediction of death

Protein	B	Wald	P-value	Odds ratio (95% CI)
Alpha-2-HS-glycoprotein	-0.161	0.143	0.706	0.85 (0.37–1.96)
Apolipoprotein A-I	-0.846	9.926	0.002	0.43 (0.25–0.73)
Apolipoprotein A-II	0.014	0.002	0.962	1.01 (0.58–1.77)
Apolipoprotein C-III	-0.159	0.513	0.474	0.85 (0.55–1.32)
Beta-2-microglobulin	0.370	1.751	0.186	1.45 (0.84–2.50)
Coagulation factor X	0.735	5.123	0.024	2.09 (1.10–3.94)
Epoxide hydrolase 1	1.147	9.875	0.002	3.15 (1.54–6.44)
Filamin-A	-1.186	11.350	0.001	0.31 (0.15–0.61)
Kallistatin	-0.285	1.225	0.268	0.75 (0.45–1.25)
Macrophage-stimulating protein	-0.198	0.626	0.429	0.82 (0.50–1.34)
Pulmonary surfactant-associated protein B	0.916	14.917	<0.001	2.50 (1.57–3.98)
Serum paraoxonase/arylesterase 1	-0.592	6.216	0.013	0.55 (0.35–0.88)

CI, confidence interval.

2.50, 95% confidence interval (CI) 1.57–3.98, $P < 0.001$], FLNA (OR 0.31, 95% CI 0.15–0.61, $P = 0.001$), APOA1 (OR 0.43, 95% CI 0.25–0.73, $P = 0.002$), EPHX1 (OR 3.15, 95% CI 1.54–6.44, $P = 0.002$), PON1 (OR 0.55, 95% CI 0.35–0.88, $P = 0.013$), and F10 (OR 2.09, 95% CI 1.10–3.94, $P = 0.024$). Associations of FLNA and SFTPB with structural and functional parameters and biomarkers are presented in the supplementary material online, Table S2.

To further confirm the value of the selected proteins, a S-plot was drafted (as presented in Figure 2) to provide a graphical representation of both the covariance and correlation structure between the selected proteins and the predictive score for death.

In linear regression analysis, these 12 proteins significantly predicted HDL cholesterol ($P < 0.001$), resulting in $R^2 = 0.579$.

When a receiver operating characteristic curve was plotted for all selected proteins combined, this resulted in an area under the curve of 0.820 (95% CI 0.768–0.872, $P < 0.001$) and a maximum sensitivity and specificity of 0.763 and 0.756, respectively (Figure 3). Internal cross validation resulted in $73.3 \pm 7.2\%$ accuracy.

Discussion

In the present study, we demonstrate that the HDL proteome is different in HF patients who survived and those who died. The strongest differences were seen in proteins reflecting crosslinking of actin filaments and alveolar capillary membrane function. These marked differences in HDL proteome composition between deaths and survivors might reveal the pathophysiological mechanisms underlying the association between HDL and clinical outcome in HF.

Previous studies investigating the HDL proteome in HF observed differences in proteome profiles between healthy subjects and HF patients and proteome profiles in HF patients associated with immune response.^{11,12} Additionally, diminished anti-inflammatory,¹³ antioxidant activity,^{13–15} and cholesterol efflux capacity¹⁶ of HDL have been implicated in HF and predicted adverse events. The present study is the first to investigate differences in HDL proteome profiles related to clinical outcome

in HF in a larger, well-characterised HF patient population with well-matched subgroups, utilising state-of-the-art nanoscale liquid chromatography-coupled mass spectrometry and powerful informatics to analyse the data.

Out of 49 HDL proteins that significantly differed between deaths and survivors, we selected 12 proteins to predict death based on multiple statistical approaches. These 12 proteins notably reflected, among others, processes related to atherogenesis, inflammation, and oxidative stress. Not much is known about their association with HF (outcome), but alpha-2-HS-glycoprotein (also called fetuin-A) levels were found to be lower in chronic HF patients,¹⁷ lower levels of APOA1 have been associated with worse prognosis in HF,^{4,5} higher beta-2-microglobulin levels have been proposed as a cardiovascular risk marker in HF,¹⁸ and decreased PON1 activity and levels have been associated with HF and adverse outcomes in HF.^{13,14,19} We will elaborate on FLNA, SFTPB, and APOC3 in the next section.

The strongest contributors to the prediction model were FLNA and SFTPB. We realise that the presence of these proteins in the HDL proteome is not direct proof for their presence in the heart, or for signalling in the heart, but nevertheless believe their presence may have functional consequences to the heart related to the associations we describe. First, FLNA is an actin-binding protein that plays a role in cell signalling functions, such as cell migration and organ development. Deficiency of FLNA is, among others, associated with severe cardiac malformation, suggesting an important function of FLNA in cardiac morphogenesis.²⁰ In a mouse model, endothelial deletion of FLNA resulted in a defective endothelial response and increased scar formation, leading to worse myocardial infarction-induced left ventricular dysfunction.²¹ These unfavourable effects of FLNA deficiency appear to be reflected by the decreased abundances of FLNA in deaths in our study.

Second, higher levels of SFTPB in deaths could be explained by the presence of increased alveolar membrane damage in a worse HF disease state due to increased pulmonary pressure, resulting in oedema and subsequently in dyspnoea in HF. Levels

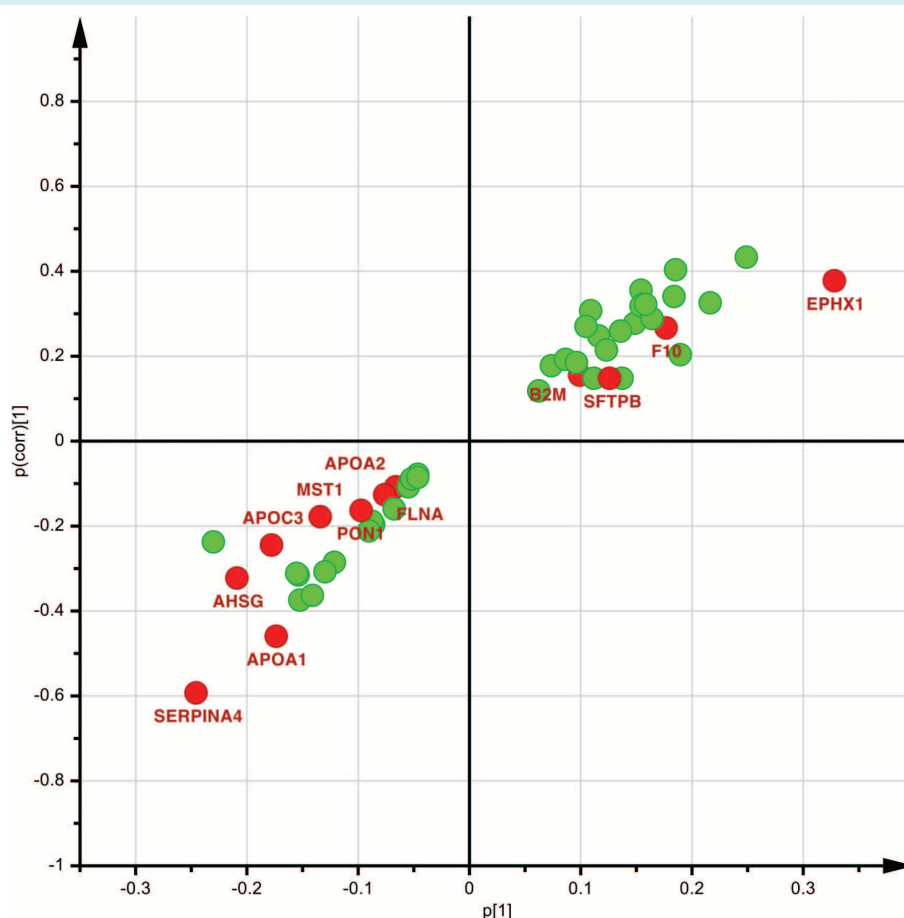


Figure 2 S-plot of high-density lipoprotein (HDL) proteins. SIMCA14 was used to generate this figure based on orthogonal projections to latent structures discriminant analysis scores for putative biomarker identification. In this figure, the p1-axis (x-axis) describes the magnitude of each variable in the data and the p(corr)1-axis (y-axis) represents the reliability of each variable in the data. The selected proteins are indicated in red while the remaining significantly different proteins between deaths and survivors are indicated in green. AHSB, alpha-2-HS-glycoprotein; APOA1, apolipoprotein A-I; APOA2, apolipoprotein A-II; APOC3, apolipoprotein C-III; B2M, beta-2-microglobulin; EPHX1, epoxide hydrolase 1; F10, coagulation factor X; FLNA, filamin-A; MST1, macrophage-stimulating protein; PON1, serum paraoxonase/arylesterase 1; SERPINA4, kallistatin; SFTPB, pulmonary surfactant-associated protein B.

of surfactant proteins in plasma, especially SFTPB, are suitable biomarkers to assess lung health and alveolar capillary membrane function, because when damaged, surfactant proteins move into the blood.^{22–24} It is thus logical for SFTPB to be higher in a worse HF disease state (reflecting increased congestion), which has moreover been shown in HF before.²⁵ Whether the HDL-bound portion of SFTPB compared to SFTPB levels in plasma is a better predictor of HF outcome is difficult to say since it is not entirely clear what portion of the total amount of SFTPB binds to HDL, but in a previous study investigating the HDL proteome in end-stage renal disease (ESRD) patients, HDL-bound SFTPB was associated with ESRD, while its plasma counterpart was comparable between ESRD patients and chronic kidney disease stage 4 patients.²⁶ It is thus possible for the SFTPB HDL-bound portion to be different from its plasma counterpart, which could possibly also be the case in different HF disease states.

Although most findings in this study are in line with the expected observations, an unexpected finding was that APOC3 levels were lower in deaths. Previous studies repeatedly showed that higher APOC3 levels are consistent with higher cardiovascular event rates due to its atherogenic effects. This has however predominantly been investigated for non-HDL lipid particles (low-density lipoprotein and very low-density lipoprotein) and total plasma; data on HDL APOC3 are too limited and inconsistent to confirm that the same is true for these particles.²⁷ However, as explained further on, a satisfactory explanation for this finding remains uncertain due to recovery of all lipoproteins by the used methodology.

Strengths and limitations of the study

This is the first study investigating HDL proteome composition in relation to clinical outcome in patients with HF. The lipoprotein isolation method that was used in this study proved to be

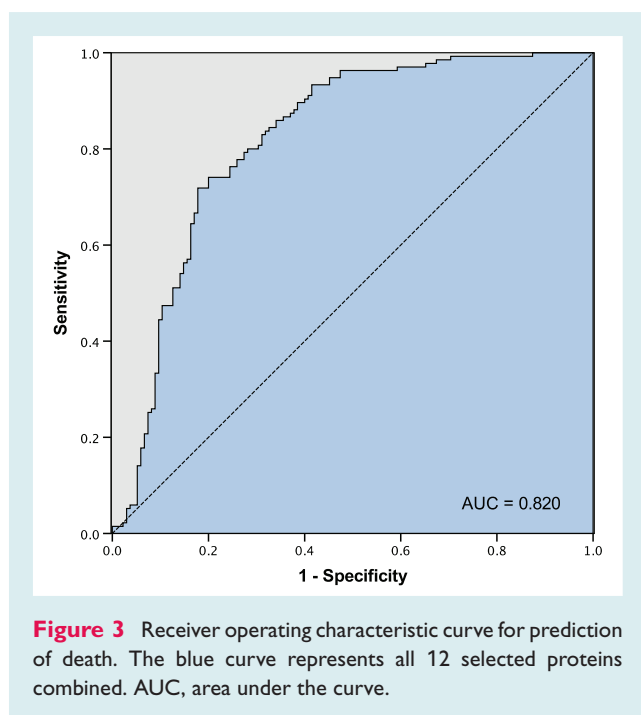


Figure 3 Receiver operating characteristic curve for prediction of death. The blue curve represents all 12 selected proteins combined. AUC, area under the curve.

highly suitable for addressing the aim of this study; we were able to detect 88.4% out of the total number of confident HDL proteins.

Furthermore, use of UPLC-HDMS^E as a means to analyse proteins ensured high selectivity, specificity and confidence. The methods of this study proved to be time-efficient, high-throughput, reproducible, capable of working with complex samples and inexpensive (both the sample preparation procedure and the label-free quantification approach). Reliability of the MS runs was ensured by running quality controls and Hela cell extract and, on top of that, use of powerful informatics to analyse the raw data. Finally, the well-matched groups ensured suitable circumstances for discovery purposes.

The fact that the survivors and the deaths groups were so well matched could however also pose a limitation: after correction for important influential factors such as age and severity of HF in a non-matched patient cohort, the HDL proteome may lose some of its predictive value. The matching used in this study cohort also limited analysis of the additive value of the HDL proteome on top of the recently constructed BIOSTAT risk score,⁶ since the groups were already matched on some of the variables present.

Also, the results of this study only apply to patients of Caucasian ethnicity and HF patients with a reduced ejection fraction. Furthermore, the lipid affinity matrix used in this study does not have specific affinity for HDL alone and other lipid subclasses could have potentially influenced the measured apolipoprotein amounts to some extent. Finally, only 49 patients had available HDL cholesterol measurements. However, we do not know whether the HDL protein to cholesterol ratio is similar for every patient, which is why there is no indication to say that this has certainly influenced our results. Furthermore, we have loaded equal total peptide amounts

on the LC-MS/MS for each sample. As a surrogate for HDL cholesterol, APOA1 is often used. We see in our analysis that APOA1 is lower in deaths in univariate and multivariate analysis, which is in line with the association between lower HDL cholesterol and adverse outcome in HF in the BIOSTAT-CHF study.

In order to address some limitations of this study, the following studies could be carried out: (i) a validation study to confirm the findings of this study in another study population; (ii) a study investigating the relationship between HDL functionality in relation with HDL proteome content and in relation to prognosis in HF; (iii) a validation of the total plasma content of FLNA and SFTPB where bound and free fractions can be calculated and independently related to outcomes; and (iv) addition of HF hospitalisation as an endpoint.

Conclusions and implications

This study shows that abundances of proteins with multiple important functions that reflect, among others, atherogenesis, inflammation, and oxidative stress, are altered in the HDL proteome of patients with a worse HF disease state. The strongest differences were seen in proteins reflecting crosslinking of actin filaments and alveolar capillary membrane function. Therefore, HDL could be a proxy for these processes implicated in HF pathophysiology which might explain the association between low HDL and increased risk of mortality and/or hospitalisation in patients with HF.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Patient matching and randomisation procedure.

Appendix S2. Protocol for MS sample preparation.

Appendix S3. Nanoscale liquid chromatography-coupled mass spectrometry.

Figure S1. Principal component analysis of HDL proteins.

Figure S2. Biological processes associated with altered HDL proteome.

Table S1. All significantly different HDL proteins between deaths and survivors.

Table S2. Correlations of FLNA and SFTPB with structural and functional parameters and biomarkers of inflammation, myocardial necrosis and wall strain.

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